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Immunity to *Candida albicans*

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INTRODUCTION

The ecological, biochemical, and morphological characteristics of *Candida albicans* make it a unique, biologically interesting microbe, and its pathological potential makes it a medically important eucaryotic microorganism. *C. albicans* can be isolated from the gastrointestinal tracts of a wide variety of birds and mammals (191); however, its association with humans is interesting from a number of viewpoints. This opportunistic yeast is a common inhabitant of the mucous membranes of humans and other animals (52, 108), and the exposure of humans to *C. albicans* occurs early in life, during passage through the birth canal (52). This yeast persists as a minor and sometimes major member of the alimentary tract microbial flora for life (57, 229).

As a consequence of colonization and persistence in the alimentary tract and through either subclinical mucocutaneous infections (107) or systemic invasion (138, 221, 234), *C. albicans* induces a hypersensitive state in a majority of healthy adults (222). In fact, a delayed hypersensitivity skin test and an in vitro blastogenesis response to *C. albicans* antigens are among the tests that clinicians routinely use to assess the cell-mediated immunocompetence of an individual (222). In addition to stimulating thymus-dependent, cell-mediated immunity, the presence of *C. albicans* in the flora also induces antibody formation in humans (228). Most procaryotic members of the microbial flora, although present in much larger numbers than *C. albicans*, do not stimulate such noticeable antibody- or cell-mediated immune responses in their hosts (15, 63).

C. albicans is also unique in the spectrum of infectious diseases that it can cause in humans and other animals (52, 128). *C. albicans* can mimic a dermatophyte infection in the skin and nails, or it can invade epithelial cells of the oral cavity, esophagus, stomach, or vagina. Even more serious clinical situations develop when *C. albicans* invades deeper tissues and causes life-threatening infections of the internal organs (228). The kidneys appear to be particularly susceptible to *C. albicans*; however, the heart, liver, lungs, spleen, brain, and eyes may all be invaded (229).

The clinical importance, biological characteristics, and classification of the genus *Candida* have been reviewed in detail elsewhere (26, 48, 189, 190, 229). Candidiasis is increasing in occurrence and severity because of advances in modern medicine and the use of antibiotic, immunosuppressive, and cytotoxic therapies and intravenous infusions (18, 20, 128, 184, 220). Unfortunately, adequate antibiotic therapy for *Candida* infections is still not available. It is important that we obtain more basic information concerning *C. albicans*-host interactions and elucidate those innate or acquired host defense mechanisms which control the diseases caused by this unique opportunistic yeast.

The purpose of this review is not to cite each report on *C. albicans* that has appeared in the literature. It was our intention to include only research that has contributed to our basic un-

derstanding of innate and acquired immunities to *C. albicans*.

INNATE AND NONIMMUNE FACTORS

Microbial Flora

One of the first eucaryotic microorganisms to come in contact with the fetus during its passage through the birth canal is *C. albicans* (205). Although this yeast causes a high incidence of mucocutaneous disease in neonates (2 to 6%), the number of infants suffering from candidiasis is less than would be expected from the increased incidence (25 to 50%) of *C. albicans* in the vagina during pregnancy (51). Most cases of infant thrush or *Candida*-induced diaper rash (205) respond to treatment or clear spontaneously and do not cause the children further problems. However, some children are not able to clear their mucocutaneous and cutaneous *C. albicans* lesions because of genetic defects or hormonal problems (51). These conditions are discussed below.

The skin and mucous membranes of humans present formidable barriers to many pathogenic microorganisms. *C. albicans* is not considered to be a normal component of the skin microflora (51, 137). Except for continuous contaminations of the surface areas immediately surrounding body openings, *C. albicans* is rarely recovered from the skin of healthy humans (137). The inability of *Candida* to persist on and colonize human skin is difficult to understand since it can utilize keratin (95), and under the right conditions of moisture (occlusion) and a proper nutritional milieu, the skins of humans (130, 164), guinea pigs (196), and rodents (163) can be invaded easily by *C. albicans*. Although other conditions are important (pH, temperature, skin shedding rate, etc.), the normal bacterial flora probably plays a role in preventing *C. albicans* colonization and subsequent invasion of skin.

Contrary to the situation on the skin, neonatal exposure to *C. albicans* results in lifelong persistence of this microbe on the mucous membranes of the alimentary tract and vagina (51). Since the bacterial flora can restrict but not eliminate *C. albicans*, this yeast is usually present as a minor component of the alimentary tract and vaginal floras. Various in vitro (82, 83, 88, 89, 234) and in vivo (8, 9, 124, 150) studies have demonstrated that intestinal bacteria can inhibit the growth of *C. albicans*. Although the exact inhibitory mechanisms have not been elucidated, nutritional competition (106), unfavorable environmental alterations (152), competition for an ecological niche (74), and the production of toxic by-products (88) contribute to the bacterial inhibition of *C. albicans* growth. Colonization of the alimentary tracts of newborns with bacteria is probably responsible for the low incidence of *Candida* disease in infants. Clinicians treating patients with oral broad-spectrum antibiotics may find that *C. albicans* quickly increases in numbers in the alimentary tract and causes disease unless nystatin or some other anti-*Candida* antibiotic is used to control overgrowth of this opportunistic yeast (184, 185).

Recent studies have demonstrated that the microbial flora in the alimentary tract may prevent gastric *C. albicans* infections in congenitally athymic (nude) mice (74). In contrast, germfree mice, rats, and chickens (all with intact thymus function) are quickly colonized and infected with *C. albicans* after oral challenge (8, 9, 150). Thus, acquired resistance to alimentary tract candidiasis may be more closely related to the acquisition of a complex bacterial flora than to antibody- or cell-mediated immunity. Disturbances in the gastrointestinal ecology through the use of antibiotics or through genetic (74, 207) or hormonal defects (106) could be an important predisposing factor in many cases of chronic mucocutaneous candidiasis and vaginitis. Further studies are needed to answer basic questions about gastrointestinal ecology and the resistance of hosts to *Candida* disease.

Hormonal Factors

In humans the pathogenicity of *C. albicans* appears to be closely associated with endocrine dysfunction and hormonal imbalances (natural or induced). Chronic mucocutaneous candidiasis (CMC) is frequently, but not always (80), associated with hypofunction of one or more endocrine organs (210). Hypoparathyroidism, adrenal failure, chronic lymphocytic thyroiditis, diabetes mellitus, ovarian failure, and adrenocorticotropic hormone deficiency have been observed individually and in various combinations with CMC (7, 16, 30, 38, 54, 75, 86, 98, 106, 109, 110, 222, 225). However, nonendocrine organ dysfunctions, such as pulmonary fibrosis (75, 225), enamel hypoplasia, keratoconjunctivitis, chronic hepatitis, pernicious anemia, alopecia totalis, juvenile cirrhosis, and the presence of antibodies against kidney tissue, also have been reported in patients with CMC (7, 75, 225). Defects in regulatory T-cells may explain the occasional occurrence in CMC patients of secretory immunoglobulin A (IgA) deficiencies and elevated levels of IgE (7). Further studies on CMC in human patients and animal models may enhance our knowledge about whether *C. albicans* is only an opportunist in such endocrine diseases or whether chronic *C. albicans* infections can trig-

ger disturbances in immunoregulation that can cause endocrine and nonendocrine organ dysfunctions.

Clinical studies have shown that females are more resistant to systemic candidiasis than males (165). In marked contrast with these findings, experimental animal studies have shown no appreciable male-female differences in resistance to experimental systemic (168) or gastric candidiasis (74). However, it is evident that vaginal colonization by *C. albicans* in humans increases with the hormonal changes and increased glycogen secretion that occur during pregnancy (22, 51, 157). The relevance of the latter change is supported by the observation that uncontrolled diabetics are also prone to develop cutaneous candidiasis (106). It is the increased concentration of glucose in the body secretions of these diabetics which is thought to play a role in their susceptibility to *Candida* disease (106).

It has been known for a long time that corticosteroid therapy decreases the resistance of a host to *C. albicans* (85, 128). Even inhaled corticosteroids have been associated with increased yeast colonization and subsequent disease of the oral cavity (202, 211). It is beyond the scope of this review to describe all of the studies that relate to the influence of hormones on immunity to infectious diseases. *C. albicans* is unique, however, in that it appears to become clinically important prior to and coincident with a wide variety of endocrine dysfunctions and hormonal imbalances that occur naturally through disease processes, with pregnancy or aging, and also iatrogenically with corticosteroid therapy. A common link among all of these diseases appears to be the increased colonization of the host with *C. albicans* before the development of *Candida* disease. An alteration occurs in the ecological balance of the microflora, which previously had curtailed the growth of *C. albicans*. Knight and Fletcher (106) have reported that an increase in the incidence of *C. albicans* occurs in the saliva of patients being treated with corticosteroids or antibiotics and in patients with diabetes mellitus. The increased concentration of glucose in the saliva allows *C. albicans* to increase in number while there is a simultaneous suppression by corticosteroids of the capacity of phagocytic cells to kill *C. albicans*. A similar sequence of hormonal changes and increased glycogen content of vaginal secretions could account for the increased number of *C. albicans* cells in the vagina during pregnancy (51). The multifaceted interactions of hormonal changes, alterations in the microbial ecologies of the alimentary tract, skin, and vagina, and disturbances in innate and acquired immune mechanisms offer exciting challenges for future research on opportunistic infections.

Phagocytosis

That aspect of the phagocytic system which functions as a part of the innate defense system is difficult to separate from the phagocytosis that is mediated (or at least aided) by acquired immune mechanisms. Since products of lymphocytes (e.g., antibody, lymphokines, etc.) can influence any or all of the fixed and circulating phagocytes, an assessment of the innate ability of a host to phagocytize and kill *Candida* is particularly difficult. It is important to realize in doing experiments on phagocytosis that experimental subjects are commonly exposed to *Candida* antigens or to similar antigens presented by other yeasts which colonize the gastrointestinal tract. This is particularly true in humans and may vary considerably in common laboratory animals. Studies have been done with "normal" human and laboratory animal phagocytes, and the wide variation and disagreement in the results of such studies may be due to unequal contributions of antigens from the flora to the immune status at the time of the experiment.

Early investigations on the phagocytosis of *Candida* were aimed at assaying the abilities of the various circulating white blood cells to take up this yeast in vitro, and these studies generally indicated some failure in killing after engulfment of the microorganism. Louria and Brayton (127) found that mouse polymorphonuclear leukocytes (PMN) were able to ingest up to seven *C. albicans* cells per phagocytic cell within 30 min. Little candidacidal activity was detected in this study, because up to 64% of the phagocytes had pseudomycelia penetrating their cell membranes after 4 h of incubation. In a similar study, Stanley and Hurley (199) found that mouse macrophages were quite capable of phagocytosis, but few *Candida* cells were actually killed. Studies aimed at quantitative determinations of the candidacidal activities of phagocytes have shown considerable variability (18.5 to 58.0%) in the percentage of the engulfed *C. albicans* cells which were killed (14, 90, 91, 115, 119, 121, 204). Leijh et al. (121) reported that although 96% of the cells in a *C. albicans* inoculum may be ingested in 1 h, up to 50% of the ingested microbes may remain viable after this period of time. Of all the circulating white blood cells, Lehrer and Cline (119) found that neutrophils had the greatest candidacidal activity.

The discrepancies apparent in these results emphasize the difficulty in evaluating the candidacidal activities of phagocytic cells when experiments are performed in vitro. An alternate assay (inhibition of *Candida*-specific amino acid

uptake and resulting macromolecular biosynthesis) has been developed (158). Peterson and Calderone (158) found that rabbit alveolar macrophages are able to inhibit 71 to 93% of the macromolecular synthesis of *Candida*. It is not clear how this inhibition relates to killing efficiency in vivo, especially in the more susceptible organs (liver and kidney); however, it does appear from such studies that phagocytosis and killing of *Candida* can take place and do occur in vivo. Be that as it may, it is most probable that some yeast cells escape phagocytic killing early in an infection and that both growth and transformation into the mycelial phase can take place. The capacity of these rabbit alveolar phagocytes to kill mycelial-phase cells has not been examined, but considering the physical size of the hyphae, one would expect that it would be more difficult for phagocytes to kill such cells.

The biochemical mechanism of killing by phagocytes has been investigated, and several candidacidal substances have been found in human neutrophils. In the presence of hydrogen peroxide (or superoxide anion) and a reactive halide, myeloperoxidase (MPO) is extremely lethal for *Candida* (105, 117, 118). Defects in the biochemistry of the fungicidal process have been described in patients with MPO deficiencies or with chronic granulomatous disease (115, 116, 118, 120, 217), and these patients have been found to be susceptible to the systemic form of candidiasis (115, 118). Neutrophils from the MPO-deficient patients possessed normal phagocytic activity, but their capacity to kill ingested *Candida* cells was much below normal. Candidastatic activity was detected in the neutrophils from patients with MPO deficiencies, but neither static nor killing activity could be detected in the neutrophils (or eosinophils and monocytes) from patients with chronic granulomatous disease (115, 118).

Diamond et al. (44, 45) have recently shown that human neutrophils possess surface receptor proteins which can bind to *Candida* pseudohyphae. The latter investigation (45) was carried out in the absence of serum, which would have eliminated any possible contribution of antibody to this binding process. Moreover, Diamond and Krezesicki (45) found that neutrophil damage in this environment was mediated primarily by oxidative biochemical mechanisms, including the MPO-hydrogen peroxide-halide system.

Evidence also has been accumulating that important alternate fungicidal mechanisms exist in neutrophils. Neutrophils from domestic chickens lack MPO, but yet they have the ability to phagocytize and kill *C. albicans*, *Staphylococcus epidermidis*, *Serratia marcescens*, and *Escherichia coli* (21). Lehrer (117) has reported that human MPO-deficient (and chronic granulomatous disease) neutrophils have the ability to kill *Candida parapsilosis* and *Candida pseudotropicalis* by a mechanism that is completely independent of MPO, iodination, and the H_2O_2 generated by the endogenous metabolism of the cell. The latter study was initiated because many MPO-deficient individuals were found to be capable of maintaining good health for prolonged periods of time.

Lehrer et al. (120) have isolated several compounds which are lethal for *C. parapsilosis* from human, rabbit, and guinea pig granulocytes. These are primarily granule- or lysosome-associated proteins, although they are not lysozyme. Peterson and Calderone (158, 159) have found that lysosome-rich materials isolated from mouse macrophages are capable of causing a reduction in both *C. albicans*-specific amino acid uptake and the number of viable yeast cells. The candidacidal mechanism described by Lehrer et al. (120) is apparently due to the cooperative effect of several proteins; the material with candidacidal activity detected by Peterson and Calderone (158, 159) has not been analyzed biochemically yet, but it resembles the antimicrobial cationic proteins isolated and characterized by Zeya and Spitznagel (235, 236).

Further evidence that neutrophils are important for defense against candidiasis has been reported by Elin et al. (58). Mice with Chediak-Higashi syndrome or a disease resembling it were found to be much more susceptible to *C. albicans* than normal mice. Humans with Chediak-Higashi syndrome have neutrophils with an impaired capacity to kill *Candida*, and they suffer frequent and severe infections with a number of microorganisms (5). In the study of Elin et al. (58) it is interesting that, although mice with Chediak-Higashi syndrome were more susceptible to *Candida*, they had circulating levels of immunoglobulin equal to or higher than those of normal mice. The amount of circulating immunoglobulin in humans with Chediak-Higashi syndrome is also normal. It should be pointed out, however, that because of the rarity of neutrophil dysfunction, extensive conclusions about the role of neutrophils in resistance against candidiasis are difficult to form. There is a significant body of evidence (see above and below) which draws attention to an association between defects in neutrophil function and the disseminated form of candidiasis. This association is of great importance when considering the contribution of each of the various host defense systems against *C. albicans*.

Innate Serum Factors

A number of serum factors with an apparent

capacity to function in the host-parasite interaction during candidiasis have been identified. It is difficult to evaluate the ability of these substances to affect the growth of *Candida* in vivo. However, it is apparent that at least some of these innate materials do contribute to overall resistance against candidiasis.

Several investigators have reported finding a substance(s) in serum which inhibits the growth of *C. albicans*. Louria and Brayton (126) have isolated a heat-stable factor with the ability to inhibit the growth of *Candida* in vitro. The structure of this material has not been determined, but it has a molecular weight of 10,000 to 20,000 and apparently contains a protein moiety(ies) since the substance(s) is sensitive to protease treatment. Later studies by Chilgren et al. (34) have shown that the material described by Louria and Brayton might actually be a "clumping" factor, which has no actual microbicidal activity.

Lorincz et al. (125), Roth et al. (174) and Roth and Goldstein (175) have described a serum component with candidastatic activity, which apparently differs from the factor of Louria and Brayton in size, heat stability, and specificity. The material characterized by Louria and Brayton (126) has surprising specificity, since it affects only *C. albicans* and *Candida stellatoidea* and not other *Candida* species, *Cryptococcus neoformans*, or *Saccharomyces cerevisiae*. It should be pointed out that more recent studies by Davies and Denning (41) indicate that human serum does not inhibit the growth or multiplication of *C. albicans*. The nature of the disagreement between the results of these studies remains to be resolved.

The presence in human serum of a material (not an immunoglobulin) with the capacity to clump *C. albicans* has been reported by numerous investigators. Chilgren et al. (34), Smith and Louria (192), and Katsura and Uesaka (96) have all described such an activity, and the possibility exists that each of these materials may actually represent the substance with lethal activity described by Louria and Brayton (126). Chilgren et al. (34) have reported evidence which indicates that the clumping activity of this factor may be inhibited when mixed with specific anti-*Candida* antibody (IgG). Smith and Louria (192) have suggested the possibility that this inhibition of clumping by antibody is due to competition for common binding sites on the fungus. Katsura and Uesaka (96) have proposed the use of inhibition of clumping as a diagnostic test for elevated antibody titers during deep-seated candidiasis.

The structure of the clumping factor remains uncertain at the present time. Smith and Louria (192) have characterized the clumping material in rabbit serum as a macroeuglobulin with fast beta electrophoretic mobility. More complete biochemical analysis of this factor would probably be of value, particularly if clumping activity is to be employed as a diagnostic tool.

Serum iron and copper have been identified as factors involved in resistance against numerous infections, including *C. albicans* infections. A number of in vitro studies have led to the identification of nutritional factors, including both iron and copper, which have the ability to modify the morphology of *C. albicans* (60, 112, 186, 233), and there is some evidence which indicates that the mycelial phase of *C. albicans* is less virulent than the yeast phase (61, 188). Vaughn and Weinberg (219) have reported that copper depresses filamentation and, in addition, that copper injections into mice increase the virulence of the mycelial form of *Candida*. Based on the results of such studies, a survey of the serum copper levels in patients suffering from chronic candidiasis seems warranted. In contrast, Elin and Wolff (57, 59) and Kirkpatrick et al. (100) have reported that iron increases the growth of *Candida* in serum and that injections of iron enhance the lethality of *Candida* for mice. Iron-unsaturated lactoferrin (an iron-binding protein) decreases *Candida* growth, but this effect is abrogated when the lactoferrin is saturated (100). There is much evidence (223) which indicates that increased serum iron levels may enhance the susceptibility of hosts to a number of infectious agents.

It is becoming more evident that at least two vitamins (vitamins A and C) are very important for resistance to candidiasis. Cohen and Elin (37) have reported that vitamin A-treated mice are more resistant (as measured by survival time) to *C. albicans* than nontreated controls. Smith et al. (193) have found that ascorbic acid reduces the capacity of neutrophils to kill *Candida* in vitro. This is in contrast to the studies of Rogers et al. T. J. Rogers, K. Adams, M. Mallon, B. Hafdahl, V. Rivas, R. Donnelly, and K. O'Day (manuscript in preparation), who found that scorbutic guinea pigs have an increased susceptibility to renal candidiasis and, furthermore, that mice receiving diets supplemented with ascorbic acid show a slight, but detectable, increase in resistance against disseminated candidiasis. Further work in this regard is presently underway.

Although commonly associated with acquired antibody-mediated immune response, serum complement levels are important factors in the arsenal of innate factors present in normal serum. Morelli and Rosenberg (146) have found that complement-deficient mice do not survive

as long after a lethal *Candida* challenge as mice with an intact complement system. These workers (147) have also shown that the complement system enhances the rate of phagocytosis, but not the rate of intracellular killing of *Candida*. The possibility that antibody may have contributed to the latter results was considered (147), and specific antibody was found to increase the phagocytosis but not the intracellular killing of the fungus. Such studies are important in light of the fact that zymosan, a component known to be present in the cell walls of many fungi, including *Candida*, is capable of activating the alternate (antibody-independent) complement pathway.

There certainly are additional serum components with the apparent capacity to modify *C. albicans* in vivo growth or in vitro growth or both; however, the degree of protection appears to be minor in comparison with the innate factors described above.

ACQUIRED IMMUNITY

Experimental Models

Workers from several laboratories have claimed success in showing acquired immunity to candidiasis in conventional mice (49, 67, 72, 73, 148, 154) by demonstrating that vaccination with viable *C. albicans* cells has a protective effect against a subsequent *Candida* challenge. Mourad and Friedman (148) and Dobias (49) have also found that vaccination with dead *Candida* cells protects mice against a lethal challenge. The latter results are contrary to the study of Hasenclever and Mitchell (72), who showed that vaccination with viable *Candida* cells is necessary to provide protection against a subsequent challenge. Such studies indicate that an increased acquired specific immune response provides mice with increased resistance to *Candida* challenge; however, a comparable degree of protection was obtained when mice were treated with bacterial endotoxin (49, 65, 72, 73, 99, 231) or sterile milk (49). Most of these agents are known to have a variety of effects on this animal, including a nonspecific stimulation of both the innate and acquired immune systems (4, 53, 92, 131).

There are reports that *C. albicans* produces an endotoxin (49, 71, 72, 87, 179) and that immunity to this endotoxin may confer overall immunity to candidiasis (71, 87, 179, 231). It now appears that the *Candida* endotoxin is not similar to bacterial endotoxin and that the toxic effects of this substance occur only at concentrations which are far above the levels that would be encountered during infections (31, 40). It is possible that injections of this endotoxin may stimulate the innate defense system and may not actually provide long-lasting specific immunity against candidiasis (40, 102).

Because *Candida* is present in the gastrointestinal tract, most people are skin test positive for *Candida* antigens. Several investigators have demonstrated that both antibody and cellular immune responses specific for *Candida* are manifested during candidiasis in various experimental animals (50, 66, 154, 171, 172, 178, 196). Marra and Balish (136) have shown that in the absence of any experimental challenge with *C. albicans*, normal mice manifest positive immediate and delayed skin test responses to *Candida* antigen, but that germfree animals do not. Rogers and Balish (171) have demonstrated that lymphocytes from conventional rats, but not germfree rats, undergo a positive *Candida* antigen-specific blastogenic response in vitro before any experimental challenge with live *Candida* cells. Several animal models have been described and are presently in use to study the role of acquired immunity in defense against infection with *C. albicans* (2, 49, 73, 93, 136, 148, 168, 196). Because most mammals are colonized with *Candida* or similar yeasts before experimental challenge and because of the evidence indicating that at least some normal animals possess positive acquired responses before any experimental manipulation, it is difficult to assess the relative roles of acquired resistance and innate resistance against candidiasis.

In an effort to circumvent this problem of prior exposure to the yeast, several investigators have attempted to characterize the susceptibility of germfree animals to candidiasis (9, 83, 160, 168, 170). Germfree animals have been reported to be more susceptible than conventional animals to a variety of infectious microorganisms (129, 160, 197, 201, 213). This increased susceptibility of germfree animals may be related to what is apparently a poorer innate defense system. Bauer et al. (11, 12) have reported that phagocytes from axenic animals have a reduced capacity to kill *E. coli* and *S. marcescens*. Abrams and Bishop (1) and Carter and Pollard (29) have reported that germfree animals mount a weaker inflammatory response than their conventional counterparts. There are also indications that several parameters of acquired immunity are weaker in germfree animals (11, 69, 79, 151, 160, 161, 171, 230), although this is somewhat controversial (19, 77, 78, 81, 201). The levels of immunoglobulin are significantly lower in germfree animals, but once an antibody response is mounted, the amplitude of the response is similar to that of conventional animals (10, 129, 230). Little is known about the acquired cellular immune response in germfree animals. Lymphoid organs in these animals are consid-

erably smaller and contain very few mature lymphocytes (11, 151, 209). Lev and Battisto (122) have reported that germfree guinea pigs have impaired delayed-type hypersensitivity responses, and this has been confirmed by others (212, 214). Jungi and McGregor (94) reported that macrophages in gnotobiotic rats had an impaired capacity for chemotaxis, but neutrophil chemotaxis, serum levels of chemotactic activity, and the capacity of lymphocytes to generate macrophage chemotactic factor were all normal. Unfortunately, the rats used in the study reported by Jungi and McGregor were not entirely germfree, but rather were associated with unidentified bacteria. Rogers and Balish (171) recently reported that lymphocytes from germfree rats do not respond to T- and B-cell mitogens in vitro as well as lymphocytes from conventional rats.

The lower levels of immune responsiveness reported for germfree animals should have a profound effect on the definition employed for innate defenses. The innate defense of a conventional animal is more potent than the innate defense of a germfree animal. Rogers and Balish (170) have demonstrated that resistance to candidiasis can be increased by an agent which nonspecifically stimulates what could be defined as the innate defense system. In this study, germfree rats were found to have a significantly higher susceptibility to candidiasis than their conventional counterparts. However, germfree rats receiving a single injection of incomplete Freund adjuvant were not more susceptible to systemic candidiasis than conventional rats. Unfortunately, the specific effects of such a treatment remain cloudy (for example, both T- and B-cell blastogenic responses were shown to be improved by treatment with this adjuvant), and so more work is needed to define adequately the contributions of innate immunity and acquired immunity to candidiasis.

Antibody-Mediated Immunity

In much of the early work on acquired immunity to candidiasis, attempts were made to stimulate the production of serum factors which would enhance host resistance to *C. albicans* infections (49). Many of the investigators failed to divulge important aspects of their experiments (e.g., the number of cells used in immunizing and challenge doses, route of immunization, route of challenge, etc.). Most of these early studies have been summarized by Dobias (49), and he too points out their deficiencies. Certain reports suggested that serum has the capacity to kill *C. albicans* in vitro (126). Other investigators found that because *C. albicans* forms germ tubes and is often agglutinated in serum, it is possible to have the false impression of a serum-mediated killing effect (34). Thus, to date there is little evidence to indicate that serum antibody and complement can kill *C. albicans* in vitro.

However, there are reports on the capacity of immune serum to passively transfer resistance to *C. albicans* in humans (76) and in experimental animals (2, 148). Mourad and Friedman (148) have demonstrated that the subcutaneous injection (total volume, 2.0 ml) of high-titered mouse anti-*Candida* antiserum substantially reduces the number of deaths normally associated with an intravenous *C. albicans* challenge. Hiatt and Martin (76) have also found that high-titer anti-*Candida* antiserum is effective in the clearing of *Candida* infections in humans.

Pearsall et al. (154) reported recently that mice injected with immune sera were more resistant to intramuscular candidiasis than control mice treated with normal serum. On the other hand, these investigators showed that adoptive transfer of immune lymphocytes had no effect against *Candida* infections (154). Al-Doory (2) has shown that baboons can be protected against *C. albicans* by intramuscular injections of immune serum from baboons that have been challenged previously with viable *C. albicans* cells.

The recent development of animal models that can selectively eliminate the contribution of the T-cell arm of immunity (39, 64, 173) may provide an opportunity to properly assess the role of antibody-mediated immunity in resistance to candidiasis. It should be emphasized, however, that the overwhelming majority of human candidiasis cases do not occur in patients with deficiencies in the capacity to produce antibodies. Furthermore, patients with candidiasis typically manifest normal (sometimes even abnormally high) levels of agglutinins and precipitins against *Candida* antigens (68, 102, 113).

The presence of an indigenous bacterial flora can interfere with the attachment of *C. albicans* to epithelial cells (124); however, a similar role for secretory IgA has not yet been described. Anti-*Candida* secretory IgA has been demonstrated to be deficient in some (33, 34, 102) but not all (114) patients with CMC. Vaginal secretions (139, 140) and sera (140) from patients with chronic *Candida* vaginitis often have increased levels of secretory IgA. At present, it appears that the increased levels of secretory IgA which occur after exposure to *C. albicans* antigens in humans or experimental animals (56, 140) are not able to prevent or eradicate oral or vaginal *Candida* infections. Thus, once again it appears that a competitive bacterial flora rather than an acquired immune response may be necessary to control the growth and infectivity of *C. albicans* in the alimentary tract and vagina.

There is another aspect of the importance of humoral factors in resistance to *C. albicans* infections that has not been explored, and this has to do with the fact that severe CMC may at times be associated with serum factors or antigen-antibody complexes that can inhibit the cell-mediated immune system (6, 28, 172). Such serum factors could interfere with the capacity of phagocytic cells to eliminate *C. albicans* from infected tissue. More work along these lines of investigation is still needed.

Cell-Mediated Immunity

Human mucocutaneous candidiasis. Studies of human CMC have led to the general belief that classical thymus-dependent, cell-mediated immunity plays a major role in resistance against disease caused by *C. albicans*. Animal studies on resistance to candidiasis have not been as conclusive, however, and the use of many dissimilar models has added to the confusion concerning the role of acquired cellular resistance against this infectious yeast (Table 1).

Resistance against human CMC has been directly related to the function of the cellular immune system. The evidence for this is, first, the association of CMC with congenital abnormalities of the thymus and thymus-related tissues. An association has been noted between CMC and a number of immunodeficiency diseases in which the cellular immune system is altered (DiGeorge and Nezelof-Allibone syndromes [47, 109] and Swiss-type agammaglobulinemia or thymus dysplasia [142, 144, 149]).

Antibody-mediated immunity in most patients with CMC appears to be totally intact, resulting in increased anti-*Candida* antibody titers in most patients and in some instances the formation of autoantibody (which is believed by some to be the cause of certain endocrinopathies often associated with CMC) (84, 102, 162, 198). CMC patients have been found to produce antibody which reacts with adrenal, thyroid, and muscle tissues, as well as thyroglobulin and parietal cells (102, 162). However, numerous investigators (23, 27, 162, 167) have reported that many patients with CMC demonstrate positive Schick tests, despite adequate diphtheria toxoid immunization. Cahill et al. (27) have reported that the failure of CMC patients to neutralize the toxin is due to a failure in the IgM-IgG switch mechanism; antitoxin antibodies of the IgM class have not been found to produce negative Schick test reactivity.

Cellular immune responses in most patients with CMC are significantly depressed. Provost et al. (162) have reported a case of CMC in which delayed hypersensitivities to a battery of skin test antigens (including *C. albicans*, mumps, streptokinase-streptodornase, and dermatophytin) were all negative. In addition, there was an inability to develop sensitivity to 1-chloro-2,4-dinitrobenzene, despite repeated sensitizing doses. The depression in cellular immune responses also included an inability of lymphocytes to produce migration inhibitory factor or to respond to *Candida* antigen in vitro.

Studies of numerous cases of CMC have led some investigators to categorize the immunoincompetence of each patient into one of four groups (216). Group I patients are not able to produce a typical hypersensitivity reaction or to synthesize normal levels of migration inhibitory factor. However, the lymphocytes from these patients do respond to *Candida* antigen in vitro by undergoing a significant increase in the level

TABLE 1. *Nature of resistance to C. albicans*

Mode of resistance	Antibody-mediated immunity	Cell-mediated immunity	Innate	Model	Type of candidiasis	Reference(s)
Specific (anti-endotoxin)	+		?	Mouse	Disseminated	49
Specific (anti-*C. albicans*)	+	−		Human	Disseminated	76
	+	−		Baboon	Disseminated	2
	+	−		Mouse	Disseminated	71–73, 148
	−	+		Mouse	Disseminated	143
		+		Guinea pig	Disseminated	178
	−	+		Human	Mucocutaneous	100–104, 114, 182, 215–217
	?	+	+	Guinea pig	Cutaneous	196
	+	−		Mouse	Subcutaneous	154
Nonspecific	−	−	+	Human	Disseminated	115–118
	−	−	+	Mouse	Disseminated	39, 67, 169, 173
		+	?	Mouse	Disseminated	226
	−	−	+	Rat	Disseminated	170

of deoxyribonucleic acid synthesis. Valdimarsson et al. (216) have reported that 20% of the CMC patients from one study could be categorized in this way. Patients suffering with this "form" of CMC have also been described by several other investigators (27, 102).

Group II, the least common group, contains patients who are unable to exhibit delayed hypersensitivity, but produce normal concentrations of migration inhibitory factor. These patients do not show the typical delayed inflammatory reaction after they receive intradermal injections of migration inhibitory factor. It has been postulated (216) that these group II patients have a monocyte dysfunction, and this is supported by the results of a study reported by Snyderman et al. (194), in which a monocyte chemotaxis defect was observed.

Patients in group III, the most common group of immunoincompetence in CMC (40% in one study), demonstrate a general defect in delayed hypersensitivity, are unable to produce migration inhibitory factor, and fail to undergo a *Candida* antigen-specific blastogenic response in vitro (216). Clark et al. (35) have reported that a defect in neutrophil chemotaxis may also be associated with group III CMC patients. The patients described in the study of Provost et al. (162) could also be included in this category. Valdimarsson et al. (216) and Paterson et al. (153) have reported that the failure of lymphocytes to undergo *Candida* antigen-specific blastogenesis is, in certain patients, due to the presence in the blood of a factor capable of suppressing the mitogenic response. On the other hand, Provost et al. (162) have reported that, at least with lymphocytes from one CMC patient, reduced antigen-specific blastogenesis occurred in the absence of a circulating suppressive factor.

Finally, group IV is made up of CMC patients with no detectable defects in cellular or humoral immunity. Valdimarsson et al. (216) have reported that up to 40% of the CMC patients in one study could be categorized in this way. The possibility that group III patients could be transformed into group IV patients has been suggested (216), but clearly more studies of group IV CMC patients are needed. It is important to point out that deficiencies in antibody formation have not been reported in any group.

The overall success of reconstructive therapy in the treatment of CMC provides additional evidence that cell-mediated immunity is involved in defense against this form of human candidiasis (23, 102). Thymus grafts (36, 123), immunocompetent lymphocytes (101–103), and transfer factor (102–104, 182, 217) have all been employed in the treatment of CMC. Transfer factor has proved to be the most remarkable treatment to date, since CMC patients in groups I, II, and III have all responded after such therapy (104, 182). In vitro and in vivo immune defects, as well as clinical symptoms, have all returned to normal after treatment with transfer factor in each of these cases (104, 182).

Experimental animal studies. It is important to point out that an adequate animal model for human CMC has not been employed in the study of immunity to experimental candidiasis. Nevertheless, a number of different models for studying immunity to candidiasis have been described (see above). Considerable controversy exists about the relative roles of cell-mediated immunity, antibody-mediated immunity, and innate immunity in defense against experimental candidiasis.

Sohnle et al. (196) have reported that resistance against cutaneous candidiasis in guinea pigs may be related to the function of the thymus-dependent cellular immune response. However, these authors (196) have also reported that completely different histopathological profiles may be obtained by varying the method of the cutaneous infection. In their guinea pig model for cutaneous candidiasis, Sohnle et al. reported that there appear to be two immune reactions to cutaneous infection; one is characteristic of the thymus-dependent cellular immune response, and the other is characteristic (on the basis of histopathological evidence) of the innate defenses. Clearly then, both acquired cellular immunity and innate defenses are involved in resistance to *Candida* in this guinea pig model, but it is still not clear whether both are involved in protection against natural cutaneous *Candida* infections. In either case, the studies of Sohnle et al. (196) have indicated that antibody-mediated mechanisms of immunity apparently do not operate in this system.

Further evidence that thymus-dependent cellular immunity may play a role in resistance to experimental candidiasis has been reported by Giger et al. (67) and Miyake et al. (143). In the study of Giger et al. (67), mice vaccinated by cutaneous infection with live *Candida* cells were found to be partially protected when challenged some time later with an intravenous dose of *C. albicans*. Thymectomized, irradiated, and bone marrow-reconstituted mice did not acquire any increase in resistance if they were vaccinated in a comparable manner. It is not possible from such studies to determine the mechanism of resistance in vaccinated mice, since both antibody and direct lymphocyte- or macrophage-mediated activities may be affected by thymectomy and irradiation. In a similar system, however, Miyake et al. (143) reported that protective immunity could be transferred (at least par-

tially) from vaccinated mice to normal recipients with lymphoid cells, but not with serum; however, a protective effect was not evident until the later stages of the infection. It is interesting that in the studies reported by Giger et al. (67), the T-cell-deprived mice were no more susceptible than normal mice to a primary intravenous challenge with *C. albicans*.

There is some evidence which indicates that at least part of the proposed anti-*Candida* effect of thymus-dependent, cell-mediated immunity may be via the elicitation of an inhibitory factor from sensitized lymphocytes. Pearsall et al. (156) have discovered a substance, apparently a lymphokine, which has the capacity to reduce the number of viable *C. albicans* cells in vitro. It should be pointed out, however, that the method of culturing the sensitized lymphocytes in the experiment of Pearsall et al. (156) included the use of nystatin, a potent antifungal antibiotic. The contribution of the nystatin to the overall anti-*Candida* activity of the lymphokine preparation is not clear at this time. Some degree of confirmation for the experiments of Pearsall et al. (156) exists in a report by Salvin et al. (180), who found that *Mycobacterium bovis* strain BCG-vaccinated mice possessed substances (apparently not antibodies) in their sera which showed potent anti-*Candida* activity in vitro. Evidence that this serum activity represents some aspect of the thymus-dependent cellular immune system is presumptive at this time, however.

Williams et al. (226) have reported data which indicate that activated macrophages may play a significant role in resistance against disseminated candidiasis. Mice treated with glucan (a β-1,3-linked polyglucose derived from *S. cerevisiae* and an agent with demonstrated abilities to stimulate the phagocytic activity of the reticuloendothelial system, as well as facets of humoral and cellular immunity) have a significantly greater resistance to challenge with *Candida* than untreated controls. Since glucan probably affects a broad range of cells in the acquired and innate defense systems, the experiments of Williams et al. (226) are difficult to interpret in terms of defining those aspects of the immune system which play a primary role in resistance.

In contrast to the work just reviewed, there are a number of investigators who have reported an inability to detect any contribution of thymus-dependent cellular immunity toward protection against experimental candidiasis. Pearsall et al. (154) have reported that repeated transfers of lymphoid cells from *Candida*-vaccinated mice to normal recipients do not improve resistance to candidiasis, even though a state of *Candida*-specific cutaneous delayed hypersensitivity is transferred by such treatment. Marra and Balish (136) have also reported that progression of disseminated candidiasis in mice cannot be correlated with the development of, or an increase in, the cutaneous hypersensitivity response. Experiments reported by Budtz-Jorgensen (24) on monkeys with experimental candidiasis of the palate are in contrast to the findings of Marra and Balish (136), since clearing of the microorganism from the palate in monkeys appears to correlate temporally with the development of cellular hypersensitivity. Thusfar however, the actual mechanism of clearance of *Candida* from the palate has not been demonstrated.

Cutler (39) and Rogers et al. (173) have found that congenitally athymic (nude) mice are more resistant than normal (euthymic) littermates to disseminated candidiasis. In addition, Rogers et al. (173) have shown that nude mice reconstituted with a functional thymus gland are just as susceptible as normal mice to systemic infections with *C. albicans*. The studies of Giger et al. (67) have shown that thymectomized, irradiated, and bone marrow-reconstituted mice are no more susceptible than normal (euthymic) mice to disseminated candidiasis. All of these findings are significant in terms of their impact on theories of the nature of resistance against the disseminated form of candidiasis. If a thymus-dependent cellular immune response is a necessary component of such resistance, then one would expect both the euthymic and the thymus-reconstituted mice to be more resistant than nude mice to infection. It should be pointed out that several recent reports have indicated that nude mice may possess abnormally high levels of activated macrophages (32, 187). Evidence for this includes data which indicate that athymic mice may be more resistant to infections with *Listeria monocytogenes*, *Brucella abortus*, and *M. bovis* (32, 187). Resistances to all of the latter bacteria are thought to be associated with T-cell-dependent macrophage activation. However, Rogers and Balish (171) have reported that activation of macrophages by vaccination with BCG does not improve resistance to disseminated candidiasis in mice, even though a similar vaccination does lead to significant immunity against infection with *L. monocytogenes*. As mentioned above, the studies of Williams et al. (226), as well as those of Salvin and Cheng (178), are in contrast to the results of Rogers and Balish (171) in that the former studies show that activated macrophages appear to be capable of effecting resistance to *Candida*. The report by Rogers and Balish (171) indicates, however, that activated macrophages, although capable of defense against candidiasis, may not express this activity

or at least be solely responsible for resistance to *Candida*. Certain findings (see below) suggest that the reason for the failure in the macrophage-mediated anti-*Candida* response may be in part anatomical.

HISTOPATHOLOGY

Histopathological examinations of the cellular infiltrates in the infectious loci are interesting in light of the various theories regarding resistance to candidiasis. In humans, the mucocutaneous form of the infection is composed characteristically of a dermal influx of chronic inflammatory cells, primarily lymphocytes and histiocytes (110, 113, 166). Plasma cells, giant cells, and occasional microabscess formation may also be observed. Hyperkeratosis, parakeratosis, and acanthosis of the epidermal layer are also characteristic (113, 166) in mucocutaneous candidiasis. In most cases the inflammatory exudate in the acute forms of the cutaneous disease is apparently mixed; that is, it is composed of intense infiltrates of PMN, histiocytes, and some lymphocytes (113, 166).

In cases of human disseminated candidiasis, microabscesses containing both intact and degenerating neutrophils surrounded by a zone of histiocytes are typical of infected tissues (128, 166). In cases where the immune system of a patient is extremely compromised, there is often a very mild or no cellular reaction at the infected site. In most cases the infectious loci demonstrate widespread edema and extensive tissue necrosis. The kidney is the most susceptible organ to disease in both human disseminated candidiasis and iatrogenic renal candidiasis after parenteral hyperalimentation (128, 166, 168).

Histopathological studies on cutaneous, intramuscular, and disseminated experimental animal candidiases have been reported by several investigators (66, 67, 93, 155, 168, 196). Giger et al. (66, 67) studied the histopathology of cutaneous lesions of mice infected intradermally with viable *C. albicans* cells. They showed that the reaction is made up of abscess formation in the deep dermal tissue, surrounded by a mixed acute and chronic inflammatory reaction. Foamy histiocytes are common in the surrounding tissue, but giant cells are not present. Reactions typical of chronic inflammation (i.e., granuloma formation) apparently have not been observed. Giger et al. (66) also reported that intradermal injections of nonviable *Candida* cells do not cause abscess formation. The histopathological composition of the lesions in animals previously vaccinated with *C. albicans* does not vary significantly from that of the lesions in nonvaccinated control animals (66). The characteristically acute inflammatory reaction just described is also observed in both thymectomized and irradiated mice (66). However, it is not clear in any of these reports at what time postinfection the histopathological examinations were made and whether there were significant changes in the cellular compositions at different sacrifice times.

Sohnle et al. (196) reported a very interesting study in which guinea pigs were infected cutaneously with *C. albicans*, either with or without occlusion of the infected site. These authors found that occluded skin lesions were characterized by a rapid accumulation of PMN, and the nature of the histopathology was not altered in animals that were previously immunized with *Candida*. In direct contrast, nonoccluded skin lesions contained a minimal PMN infiltration at 24 h, followed in immunized animals (but not in nonimmunized animals) by a mononuclear cell infiltrate in the dermis. The nature of the histopathology in the latter animals was apparently dependent on activation of the cellular immune system since nonimmune animals given lymphocytes from sensitized pigs also exhibited a mononuclear cell response.

Sohnle and Kirkpatrick (195) have shown that the lesions resulting from infections under occlusive dressings are characteristically infiltrated with large numbers of basophils. The presence of basophils is apparently independent of the immune status of the animal, since both *Candida*-immunized and nonimmunized control animals exhibit this reaction. Moreover, neither dead *Candida* cells nor a preparation of *Candida* growth filtrate is capable of inducing the basophil infiltrate. Since the basophilic infiltrate does not appear to be dependent on the presence of complement or serum antibody, Sohnle and Kirkpatrick (195) suggested that the mechanism of chemotaxis for the basophils is apparently nonimmune or at least of a nature which is still unrecognized. The role of the basophils in controlling the infection has not been determined from these or other studies, however.

Pearsall and Lagunoff (155) described a thigh muscle candidiasis model in mice, and histopathological studies of these lesions showed little evidence for an intensive thymus-dependent, cell-mediated immune response. Temporal analysis of the histopathology showed that after an initial PMN response, there was a modest infiltration of the lesion with macrophages, eosinophils, and lymphocytes. Neutrophils remained the predominant cell type present in the cellular infiltrate, however, even after the muscle began to return to normal size. A predominance of mononuclear cells was not observed until very late in the infection, when few *Candida* cells

remained and the muscle tissue histology had returned to normal.

Pearsall and Lagunoff (155) also indicated that a muscle infection may lead to extensive amyloidosis in the animal, a result which also has been observed after injections of dead cells. More recent work by Mann and Blank (134) has supported these results. The use of animals treated in this manner could be useful as a model for the study of systemic amyloidosis.

A number of authors have described the histopathology of disseminated candidiasis, but in most cases this has been limited to the renal form of the disease. Williams et al. (226) and Rogers and Balish (unpublished data) have reported an inability to detect significant histopathology in the livers of mice with progressing renal candidiasis. This is interesting in light of the fact that, after the kidney, the liver is apparently the primary focus of infection (168). In the studies reported by Williams et al. (226), there was an absence of fungi stained by periodic acid-Schiff reagent, and there were few inflammatory cells. Previously, it was found (T. J. Rogers, Ph.D. thesis, University of Wisconsin, Madison, 1976) that the livers of mice with renal candidiasis had remarkable deposits of material stained by periodic acid-Schiff reagent, which coated cells which emanated outward from blood vessels. The nature and origin of this material stained by periodic acid-Schiff reagent is unknown at the present time, although it may represent partially digested fungi which have been filtered out by the liver. We have scanned other organs in mice infected with a sublethal dose of *Candida*, and by 7 days after infection we have been unable to detect any significant histopathology in any other organ. Of course, it is possible to culture *Candida* from other organs, in particular the liver, but apparently these microbes are not easily observed by histological techniques.

The renal histopathology has been described in great detail by several investigators, and it is clear that the tissue reaction goes through definite changes at varying times after challenge. Winblad (227), Rogers and Balish (169, 170), and Williams et al. (226) have examined tissue sections during the first 7 to 10 days after systemic challenge and have observed large abscesses, primarily in the cortex, and a more generalized inflammation by PMN, with very few mononuclear cells. It has been pointed out (226) that the reaction to infection during the first week is not intense, even though the number of microorganisms may approach 10^5 cells per kidney (168). We have suggested that this failure of host cells to infiltrate the locus of the infection rapidly is a possible explanation for kidney susceptibility to candidiasis.

By the end of the second week after challenge, the primary locus of infection has moved to the renal pelvis, and the surrounding medulla and renal cortex are heavily infiltrated by numerous inflammatory cells. We have reported previously (169, 170) that the kidney remains free of any intense mononuclear infiltrate, although a diffuse influx of macrophages and lymphocytes can be observed. In contrast to these results, however, Winblad (227) and Williams et al. (226) have reported that granuloma formation in the renal cortex does occur and may begin as early as day 7 after challenge. Nevertheless, by the end of the second week the pelvis is heavily packed with mycelial and yeastlike forms of *Candida*. Although initially the response is not intense, Rogers and Balish have reported that the cellular response to this pelvic infection is limited to the PMN and that few mononuclear cells are evident even as late as 24 days after challenge. If thymus-dependent, cell-mediated immunity is responsible for clearance of this infection, one would expect a more intense mononuclear infiltrate, especially at 3 weeks postinfection, when the number of viable microorganisms is past its peak (day 17) and is on the decline. We have observed that the fungal bolus in the pelvis at days 21 to 24 after challenge appears to be regressing and that degenerating mycelial filaments are very evident. However, with present information it is impossible to rule out the possibility that the appearance of these degenerating mycelia is purely artifactual. Nevertheless, at a time when the numbers of viable *Candida* cells are beginning to wane, the cellular infiltrate is still almost exclusively polymorphonuclear.

It is important to ascertain the degree of basophil and eosinophil influx into the kidney during the infection, especially in light of the study of Sohnle and Kirkpatrick (195). Conventional tissue fixation techniques and staining procedures are particularly hard on these cell types, and special methods must be employed in order to achieve an accurate picture of the true cellular composition. We have attempted to characterize the histopathology of renal candidiasis (169, 170), as well as liver and spleen (Rogers, unpublished data) candidiasis, by using procedures which optimize the appearance of basophils and neutrophils. Our results have indicated that few of these cells become evident during the course of a renal infection (up to day 24) (169). The occurrence of a significant basophil response, as reported by Sohnle and Kirkpatrick (195) in guinea pig cutaneous candidiasis, is not apparent

in rodent kidneys, and it may occur only in cutaneous infections.

The failure to detect mononuclear cells at the primary locus of the infection has important implications for those who propose that thymus-dependent cellular immunity is the primary factor in resistance to disseminated candidiasis. Important questions about the nature of the immune response must be raised, particularly in view of the chronic nature of the infection. It has been pointed out that the renal pelvis may be a poor environment for macrophage action, and this may be an important aspect of the pathogenesis of the infection (169, 227). Nevertheless, important questions concerning immunity to candidiasis remain. Is there an adequate induction of macrophage chemotaxis and activation during renal candidiasis? Our results indicate that, for as-yet-undefined reasons, macrophages are not present in significant numbers in the renal pelvis. If macrophages are induced and if they do infiltrate the locus of the infection, are they effective in controlling the infection? The results of Williams et al. (226) with glucan-treated mice indicate that macrophages could be highly effective. In marked contrast, we have shown previously that BCG vaccination does not improve resistance to candidiasis (169). The nature of the contradiction between these two findings remains to be resolved.

IMMUNOSUPPRESSION BY THE INFECTION

The concept that microbial infections may lead to suppression of the acquired immune system is not exactly new, since a number of microbial agents are known to be capable of acting in this way (183). *Candida* is not unusual in this regard. Several investigators have found that *C. albicans* is effective in inhibiting the activity of the immune system. Scherr (181) and Fischer and Horbach (62) have reported that injection of killed *C. albicans* cells leads to a dramatic increase in the susceptibility of mice to later challenge with live organisms. These results are somewhat controversial in view of the more recent reports by Hasenclever and Mitchell (71), Isenberg et al. Berkman (87), and Dobias (49), which indicated that vaccination with dead *Candida* cells improves resistance against candidiasis.

Rzucidlo et al. (176, 177) have prepared extracts of *C. albicans*, called zymosan, which are capable of causing significant increases in the susceptibilities of mice to infections with *Salmonella typhosa* and *Staphylococcus aureus*. Unfortunately, very large quantities of this material were used in these studies, and suitable controls for doses of cell wall material this large are particularly difficult to set up. However, similar work has been carried out by Yamabayashi (232), and in this case zymosan increased the susceptibility of hosts to infections with *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *E. coli*. No difference in resistance was observed when the zymosan treatment was followed by infections with *S. aureus* and *Streptococcus haemolyticus* (232). It is of particular importance that the zymosan effects have been shown to be very short-lived, appearing within 30 min and lasting only about 12 h.

Mankiewicz et al. (132, 133) have found that guinea pigs treated with a polysaccharide extract of *C. albicans* show a modest increase in susceptibility to *Mycobacterium tuberculosis* infections, as measured by the percentage of fatalities after a dose less than the 50% lethal dose and by the rate of tissue invasion by the microbe. The nature of this effect on resistance against *M. tuberculosis* infections is not clear, but it has been suggested that polysaccharide extracts of *Candida* may contain an endotoxin-like substance. In reviewing these results and in reporting results of experiments in which similar effects were observed in mice treated with a *Candida* extract and infected with *Salmonella enteritidis*, Dobias (49) has suggested an alternative explanation. The *Candida* extract may have contained materials which simply improved the nutritional environment for the infecting microorganisms. More work is necessary to determine the chemical nature of the active components in the *Candida* extract; in addition, the effects of other microbe extracts prepared in a similar way should be examined.

Studies carried out on animals challenged simultaneously with *C. albicans* and other microorganisms have been reported by several investigators, and it is clear that resistance against either microorganism in such circumstances is often much lower than resistance against either microorganism alone (65, 133, 181, 232). It is difficult to construct experimental controls and to analyze the results of such experiments properly, but the prospect that infection by *C. albicans* might lead to a depressed state of host immunity is certainly one conclusion which can be drawn from such work. Many of these studies have been reviewed very adequately (49), and further mention of these results is beyond the scope of the present paper. However, more recent work, not included in the review of Dobias (49), deserves some mention.

Work performed on the cellular immune status of patients with the mucocutaneous form of candidiasis (see above) has led certain investigators to the conclusion that CMC and sup-

pressed thymus-dependent, cell-mediated immunity occur concomitantly. This raises an obvious question about the cause and effect relationship. Which arises first, the immune defect or the mucocutaneous infection? If indeed the cellular immune dysfunction of CMC patients is the result of the *Candida* infection, then treatment to irradicate the disease should alleviate the immunodepression. Some evidence for this exists, including studies reported by Paterson et al. (153), Kirkpatrick et al. (2), and Budtz-Jorgensen (5). The results of the study of Budtz-Jorgensen are of particular interest in that 13 patients with candidiasis of the palate completely cleared the *Candida* disease and recovered normal cellular hypersensitivity after amphotericin B treatment. It should be pointed out that similar work by Provost et al. (162) is in marked contrast to the studies just mentioned, and, in addition, Kirkpatrick et al. (102) have pointed out that amphotericin B treatment tends to have only very temporary effects on many patients with CMC. The prospect that an infection by *Candida* may in some way contribute to a preexisting immunodeficiency cannot be ruled out, however.

We (171, 172) have reported evidence which indicates that infection of mice with *C. albicans* reduces the in vitro activity of T-cells but not B-cells, as judged by the responses of lymphocytes from infected animals to phytohemagglutinin, concanavalin A, and lipopolysaccharide. The suppression of T-cell function is not observed in rat lymphocytes until day 3 postinfection and lasts until about day 9 (171). In mice, the effect is observed by day 7, with a return to normal on day 14 (172). Studies also have been carried out on antigen-specific blastogenesis in infected animals, and suppressed T-cell responses have been observed here as well (171, 172). Moreover, BCG-vaccinated mice challenged with *Candida* demonstrate a suppressed purified protein derivative-induced blastogenic response (172). The nature of this T-cell-specific suppression is not clear, but one explanation is that T-suppressor cells or macrophages with suppressor activity are generated by the infection (for a limited period of time) and that these cells depress the response of other T-cells in culture. Direct evidence for this is presently unavailable, but such an explanation would be consistent with the data just described. Additional studies by us (171) have indicated that germfree animals do not demonstrate suppressed T-cell responses after *Candida* infections. The hypoactivity of the immune system in germfree animals has been well documented (11, 69, 79, 151, 160, 161, 171, 230), and these animals would be expected to have low levels of competent regulatory T-cells at the time of challenge. Recently, Mattingly et al. (141) have shown that T-suppressor cell activity in germfree animals is significantly depressed. These experiments are important also because they indicate a role for the host in T-cell response suppression, as we have reported (171, 172).

Recent work (Rogers, manuscript in preparation) on mice challenged with killed *Candida* cells has produced results which indicate that T-cell-specific suppression may in fact be due to the function of two independent cell populations. These two populations (a splenic T-suppressor cell population, and a macrophage population) are both necessary in order to observe any suppressor effects. It should be pointed out that Stobo et al. (200) have recently reported the finding of a putative suppressor T-cell population present in the peripheral blood of group III CMC patients. The T-suppressor cells in this case are capable of reducing phytohemagglutinin-, concanavalin A-, and *Candida* antigen-induced mitogenic responses. Additional evidence that *C. albicans* induces a state of T-lymphocyte suppression has been reported by Vardinon and Segal (218). These investigations have shown that the antibody response to T-dependent antigens, but not T-independent antigens, is depressed during experimental candidiasis in mice.

Clearly, more studies are necessary to determine the nature of the *Candida*-induced suppression of cellular immune activity. This suppression is difficult to conceive of, since most mammals are colonized by *Candida* or similar yeasts, yet a significant body of data exists which indicates that *C. albicans* may in some way, perhaps through the induction of T-suppressor cells or macrophages, reduce the overall activity of the cellular immune system.

CONCLUSION AND SPECULATIONS

It has not escaped our attention that resistance to candidiasis and resistance to other fungal infections might share common mechanisms of action. Indeed, it is tempting to speculate that a mechanism such as antibody-dependent cellular cytotoxicity, which has been suggested as a mode of resistance against cryptococcosis (43, 46), might be critical in the defense of a host against candidiasis. Evidence that antibody-dependent cellular cytotoxicity is involved in defense against candidiasis has not been forthcoming, but this mechanism cannot be ruled out as a possibility at this time. An organism as large as a fungus undoubtedly presents problems to a host which are not common to the more completely characterized and often more easily phagocytized bacteria. However, the mechanisms of resistance against other fungi have not

remained as much a paradox as the mechanisms of resistance to candidiasis. At the present time the literature seems to indicate that thymus-dependent, cell-mediated immunity plays a primary role in resistance against both histoplasmosis and coccidiomycosis (3, 13, 42, 208). Clearly, more research is necessary to clarify certain problems associated with the mechanism of resistance against candidiasis.

We have tried to review the most recent research dealing with the various aspects of host defense which may play a role in resistance against candidiasis. It is clear that much disagreement exists with respect to the relative importance of the innate and acquired defense systems. Recently, Drutz and Graybill (55) categorized candidiasis as typical of those infections in which both antibody-mediated immunity and cell-mediated immunity are decisive for recovery. Clearly, there must be more than one single mechanism for resistance against candidiasis, but it is our opinion that certain aspects of this host-parasite interaction are unique. First, resistance against the mucocutaneous form of candidiasis clearly rests with the thymus-dependent cellular immune system. Little evidence exists to the contrary, and because of the wide range of data in existence, support for this contention is convincing to say the least. However, there is little evidence which indicates that resistance against the disseminated form of candidiasis is of the same nature. Evidence from humans and much of the experimental animal evidence indicate that thymus-dependent, cell-mediated immunity is not critical for resistance against this manifestation of *Candida* disease. Is it not possible, then, that resistance against the mucocutaneous form is mediated by cell-mediated immunity and that resistance against systemic candidiasis is dependent on antibody-mediated or innate mechanisms? Based on the results of our own experiments, we contend that resistance against renal candidiasis is mediated by the innate defense system, in which neutrophils slowly and methodically eliminate the microorganism from its primary locus of infection, the renal pelvis.

It is also our contention that the host-parasite interaction during candidiasis is interesting because of the apparent effect that *C. albicans* has on the ability of the immune system to respond in general. Evidence indicates that during the course of human, rat, and mouse candidiases, immunosuppression takes place, and this is very likely (although still not proven) to be due to the stimulation of T-suppressor lymphocytes or macrophages with immunosuppressive capabilities.

Although certain aspects of the host-parasite interaction during candidiasis have been studied in great detail, research in this area should continue to provide rewarding results. We clearly have little understanding of the broad range of phenomena which occur during this type of infection. We hope that further research will provide much-needed answers to important clinical questions.

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